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Microbiological Assay of Different Brands of Fluconazole capsules retailed in Abuja, Federal Capital Territory, Nigeria.

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ABSTRACT

Candidiasis caused by C. albicans is the most commonly reported opportunistic infection observed in HIV/AIDS patients, occurring in an estimated 80–95% of those with HIV disease. Six different brands of fluconazole hydrochloride marketed in Abuja, Nigeria were collected and assayed using agar diffusion method against clinical isolate of Candida albicans. For the purpose of comparism reference fluconazole powder was also screened. The diameter of zones of inhibition produced by the different brands of fluconazole against C. albicans ranged between 22.67 – 29.67 mm. Five out of the six brands of fluconazole capsules assayed were comparable with the reference standard.

Key words: *fluconazole, agar diffusion, zone of inhibition.*

INTRODUCTION

The use of antifungal agents has increased greatly due to the increasing number of *C. albicans* infections. A consequence in clinical practice of the wide use of azoles is the frequently encountered isolates resistant to these agents [1, 2]. Given the widespread use of this agent, concerns about the development of resistance in yeast have been raised [3]. *Candida albicans* is the most common fungal pathogen and is responsible for majority of localized fungal infections in humans [4]. It is a commensal fungus and an important opportunistic pathogen causing ailments such as thrush, vaginitis and invasive infections in immunocompromised patients. Candidiasis is the most commonly reported opportunistic infection observed in HIV/AIDS patients, occurring in an estimated 80–95% of those with HIV disease [2]. Fluconazole, a triazole agent, is one of the most commonly prescribed systemic antifungals [5]. It is well absorbed after oral administration and shows good penetration into cerebrospinal fluid [6]. This agent is used in the treatment of oropharyngeal, esophageal, or vulvovaginal candidiasis, as well as other serious systemic candida infections. It is also used for the treatment of meningitis caused by *Cryptococcus* sp. [7].

There has been an increasing infiltration of fake and sub standard drugs into the Nigerian market. It is therefore important to periodically perform quantitative and qualitative assessment of drugs in the market, as capsules with dosage lower than that recommended are ineffective and compromise patient health. The aim of this study is to assess the microbiological quality of six different brands of fluconazole capsules marketed in Abuja, Nigeria.

MATERIAL AND METHODS

Reagents and materials:

Fluconazole powder reference standard was purchased from Sigma- Aldrich (USA, F8929). Fluconazole capsules (150 mg) were obtained from different pharmacies in Abuja, Nigeria. Distilled water purified in a Millipore (Bedford, MA, USA) system was used in the analysis and dimethyl sulfoxide (DMSO) (analytical grade) from Sigma.

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Preparation of inoculums:

Candida albicans was maintained on Sabouraud dextrose agar slant at 4 °C. Prior to use, the microorganism was transferred to Sabouraud dextrose slant agar and incubated for 24 h at 28 °C. After this period, a small portion of the yeast was transferred to a sterile saline solution (0.9%) and adjusted to 0.5 Mc Farland standard [2]. Further dilution of the inocula suspension was made in 15 mL of Sabouraud dextrose agar melted at 45 °C to achieve a final concentration of 1-5 x 105 CFU/mL.

Bioassay of fluconazole:

A modification of agar well diffusion method was used as recommended by Queiroz *et al*., 2009 [8]. A stock standard solution of 1000 µg/mL was prepared by placing 10 mg of fluconazole reference standard into a flask and diluting in DMSO to a total volume of 10 mL. This solution was further diluted in sterile distilled water to a concentration of 156.25 µg/mL. A pool of each sample of fluconazole capsules (F1, F2, F3, F4, F5 and F6) were made and a stock solution of 1000 µg/mL in DMSO was prepared for each of the samples. This solution was diluted in sterile distilled water to a concentration of 156.25 µg/mL (identical to the standard reference concentration - R). All the fluconazole solutions (standard and test) were prepared immediately before performing the test. Sterile petri dishes (100 mm x 20 mm) were used in all microbiological tests. A base layer of Sabouraud dextrose agar (8 mL) was plated before the test to facilitate the visualization of inhibition zones. After base layer solidification, the next layer, to be used for inoculation, was poured into petri dishes on top of the base layer. The agar was allowed to solidify at 25°C for 10 to 15 min and 6 mm-diameter wells bored on the plates. The reference concentration (R) was tested concomitantly with each sample concentrations. Hundred micro liters of each standard or test solution were pipetted into individual wells. The plates were incubated at 28 – 30 °C for 24 h and the diameter inhibition zone measured using calipers. Assay plates were tested in triplicate.

Statistical analysis:

Results obtained were expressed as mean ± standard deviation and analysed using one way ANOVA (Smith's Statistical Package version 2.80) at $p < 0.05$.

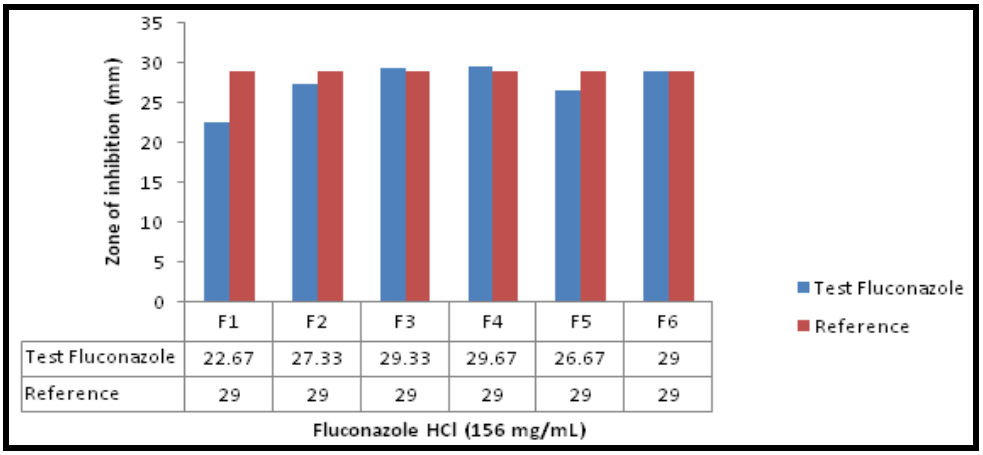
RESULTS AND DISCUSSION

Five out of the six brands of fluconazole hydrochloride assayed showed diameter zones of inhibition comparable with the reference ($p > 0.05$). The diameter of zones of inhibition (22.0 – 29.3

mm) of the test fluconazole samples alongside the reference is represented in **Fig. 1**.

The use of fluconazole hydrochloride capsules is wide spread around the world, as the pharmaceutical industries and compounding pharmacies produce the capsules for human usage, hence capsules with active pharmaceutical ingredients (API) content lower than recommended compromise patients health and is unfit for use. Given the widespread use of this agent, concerns about the development of resistance in yeast have been raised [9]. The results of the bioassay revealed a significant difference ($p<0.05$) in the diameter zones of inhibition between sample F1 (22.67 ± 0 577 mm) and the reference drug R (29.0 ±0.0 mm). This reduction in the zone of inhibition may have a negative impact on the efficacy of the drugs against the microorganisms. Research findings have linked therapeutic failure of some drugs marketed in Nigeria to the presence of insufficient or lack of active ingredients [10]. It is also note worthy that the quantity of API is not the only factor that can affect the efficacy of medicines. Formulation excipients and methods are factors of consideration in the release and dissolution profile of the API, this in turn reduce their therapeutic effectiveness.

The other brands of fluconazole assayed showed slight variability in diameter zones of inhibition in comparism with the reference, however, the differences in terms of millimeters were not statistically significant ($p>0.05$). This could be linked in the uniformity of the contents of the different brands of fluconazole and reference drug.



	F1	F2	F3	F4	F5	F6
Test Fluconazole	22.67	27.33	29.33	29.67	26.67	29
Reference	29	29	29	29	29	29

Fig. 1: Zone of inhibition of diffrent brands of fluconazole capsules against *C. albicans*

CONCLUSION

From the results of the bioassay, five out of the six brands of fluconazole capsules assayed produced diameters zones of inhibition comparable with the reference fluconazole.

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